American Journal of Hematology 56:183–186 (1997)

Severe Thrombocytopenia Suggesting Immunological Mechanisms in Two Cases of Vivax Malaria

Sayuri Yamaguchi,[1] Tatsumi Kubota,[1] Takahiro Yamagishi,[1] Kiyoshi Okamoto,[1] Tomoyuki Izumi,[1] Masashi Takada,[1] Shigeyuki Kanou,[2] Mamoru Suzuki[2], Jun Tsuchiya,[3] and Takuji Naruse[4]

[1]Department of Internal Medicine, Fukaya Red Cross Hospital, Saitama, Japan
[2]Department of Parasitology, Gunma University School of Medicine, Tokyo, Japan
[3]Gunma University School of Health Sciences, Tokyo, Japan
[4]Department of Third Internal Medicine, Gunma University School of Medicine, Tokyo, Japan

Case 1: A 27-year-old woman, referred to our hospital because of relapsing fever after travel to Thailand, was given a diagnosis of vivax malaria. Clinical investigation revealed thrombocytopenia, elevated platelet-associated IgG (PAIgG), and negative antibody against *Plasmodium vivax* antigen. After antimalarial treatment, the levels of both the platelets and PAIgG returned to normal. Case 2: A 28-year-old Sri Lankan man was admitted to our hospital with a complaint of fever. The patient had thrombocytopenia, elevated PAIgG, and positive antibody against *Plasmodium vivax* antigen. He contracted malaria in Sri Lanka about 6 months prior to this admission. After treatment, the platelet count and PAIgG level returned to normal. In these two cases, high levels of PAIgG may have been involved in the development of the thrombocytopenia. In the first patient, in particular, the thrombocytopenia was thought to be induced by some immunological mechanism prior to the detection of antimaralial antibodies in serum. Am. J. Hematol. 56:183–186, 1997. © 1997 Wiley-Liss, Inc.

Key words: vivax malaria; platelet-associated IgG (PAIgG); thrombocytopenia; antimalarial antibody

INTRODUCTION

The number of malaria patients in Japan has been increasing recently, because more travelers are going abroad to destinations such as Southeast Asia, South America, and Africa, and more foreigners are entering Japan from countries where the incidence of malaria is high [1]. Thrombocytopenia is a common findings in both *Plasmodium* (*P.*) *vivax* and *P. falciparum* malaria [2,3], but the mechanism of thrombocytopenia in malaria remains unknown. We report here two cases with malaria vivax who showed thrombocytopenia suggesting an immunological mechanism and increased level of platelet-associated IgG (PAIgG).

CASE REPORTS

Case 1

A 27-year-old Japanese woman was admitted to our hospital because of a fever after an 11-day trip to Thailand. Ten days after she returned to Japan, she developed a 40°C fever, which vanished the next day. On the third day, the fever occurred again, and the patient was admitted to our hospital. Her physical examination was unremarkable except for the presence of fever. Laboratory investigations were performed: white blood cell count 56.0×10^9/l, with 38% neutrophils, 52% lymphocytes, 6% monocytes, and 4% atypical lymphocytes; hemoglobin 11.5 g/dl and platelet count 22×10^9/l. Blood chemistries disclosed elevated LDH (307 IU/l (normal 60–22 IU/l)), total bilirubin 1.2 mg/dl, direct bilirubin 0.2 mg/dl, CRP 16.35 mg/ml, haptoglobin <10 mg/100 ml, Coombs test (direct, indirect) negative, complement 37.3 CH50U/ml and immune complexes (C1q) <1.5 μg/ml. Blood coagulation test revealed no abnormality for disseminated intravascular coagulation (DIC). The PAIgG

*Correspondence to: Dr. Sayuri Motomura, Department of Hematology, Tokyo Women's Medical College, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo, Japan.

Received for publication 4 April 1997; Accepted 11 June 1997

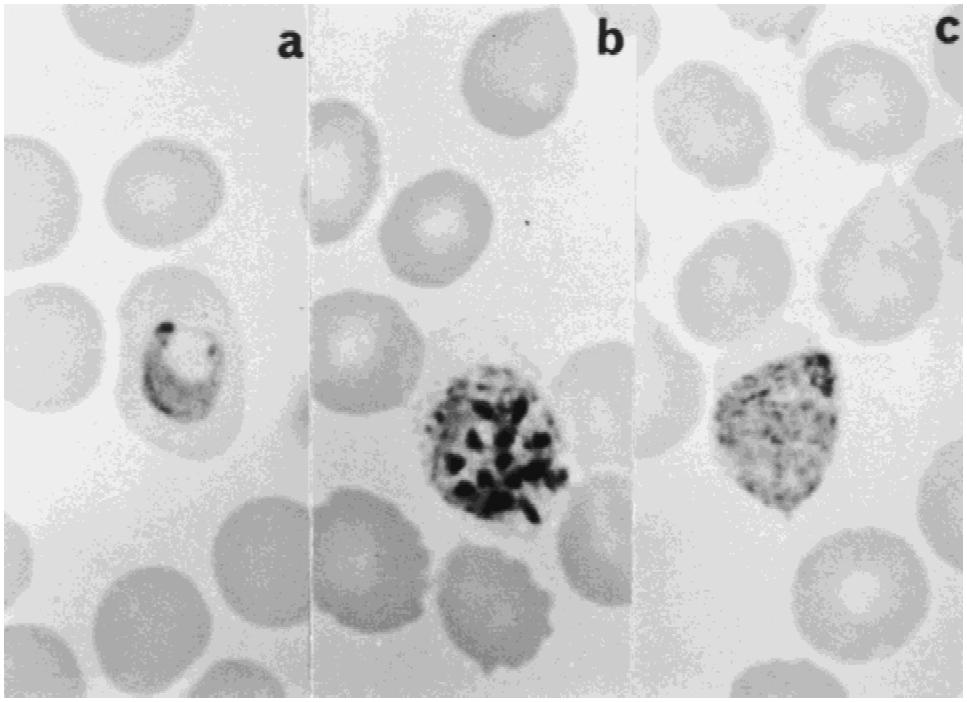

Fig. 1. Trophozite (a), shizont (b), and gametocytes (c) of *P. vivax* in case 1 (Wight-Giemsa stain, ×1,000).

level was elevated to 308 ng/10^7 cells (normal 9.0–25.0 ng/10^7 cells). The bone marrow aspiration disclosed normocellular bone marrow with normal numbers of megakaryocytes. Ultrasonography of the abdomen revealed mild splenomegaly. Peripheral blood smear revealed enlarged red blood cells (RBC) including the gametocytes, shizont and trophozoite of *Plasmodium vivax* in 0.6% of the RBC (Fig. 1). Antibody titers against *P. vivax* and *P. falciparum* were negative, although after 3 days those against *P. vivax* were found to be positive at 1:4 by indirect fluorescent antibody test [4,5]. The patient was treated with sulfadoxine (25 mg)-pyrimethamine (500 mg) (Fansidar) 3 tablets, followed by primaquine at 15 mg per day for 14 days (Fig. 2A). On the fourth day of treatment, the fever vanished and *P. vivax* was not seen in the blood smear. The thrombocytopenia and increase of PAIgG were no longer present by the eighth and twelfth day, respectively.

Case 2

A 28-year-old Sri Lankan man was admitted to our hospital because of a fever. He had come to Japan 4 years earlier, and had gone to Sri Lanka 8 month prior to this admission; he was infected with malaria during that trip to Sri Lanka. He had a 39°C fever in January, February, and March for 3 to 7 days each time. He did not seek treatment at the time, because the fever was subsided within one week. The patient had a 39°C intermittent fever (3–5 days) again in April, at which time he was admitted. His physical examination was normal except for mild icterus and fever. Laboratory investigations were performed: white blood cell count 36.0 × 10^9/l, with 44% neutrophils, 34% lymphocytes, 16% monocytes, 1% eosinophiles, and 5% atypical lymphocytes; hemoglobin 10.8 g/dl and platelet count 53 × 10^9/l, LDH 334 IU/l, total bilirubin 1.6 mg/dl, direct bilirubin 0.4 mg/dl, CRP 16.33 mg/ml, haptoglobin 39 mg/100 ml, Coombs test (direct, indirect) negative, complement 41.5 CH50U/ml, and immune complexes (C1q) <1.5 μg/ml. The blood coagulation test result was normal, and the PAIgG level was 431.7 ng/10^7 cells. The bone marrow contained normal numbers of cells and megakaryocytes. Ultrasonography of the abdomen revealed mild splenomegaly. Peripheral blood smear revealed enlarged RBC including *P. vivax* in 2% of the RBC. Antibody titers against *P. vivas* and *P. falciparum* were shown at 1:256 and 1:64, respectively. The patient received chloroquine 150 mg per day for 3 days, followed by primaquine at 15 mg per day for 14 days (Fig. 2B). The fever and *P. vivax* in blood smear vanished on the third day. The thrombocytopenia was no longer present on the seventh day, and the PAIgG level was decreased to 111.5 ng/10^7 cells on the seventh day. This case was considered to be a recurrence of the malaria contracted in Sri Lanka.

DISCUSSION

Thrombocytopenia is a common pathological feature of malaria [2,3]. It is reported that 22 of 26 (85%) patients with falciparum malaria and 30 of 39 (72%) patients with vivax malaria had depressed platelet counts below 150 × 10^9/l [6]. The mechanism of thrombocyto-

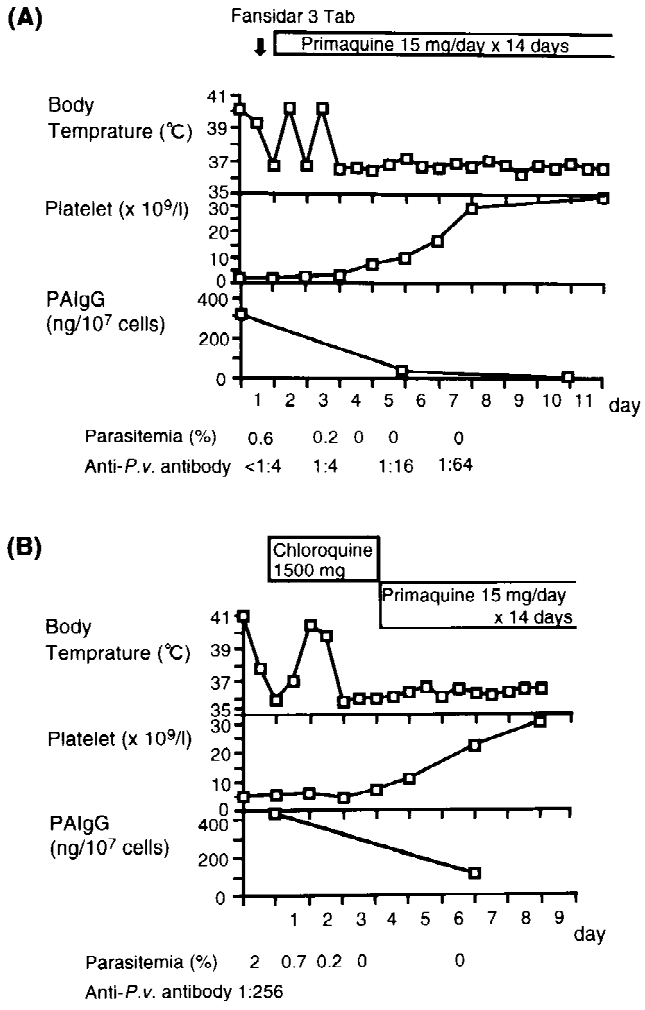

Fig. 2. Clinical course of case 1 (A) and case 2 (B).

penia in acute malaria remains unknown. It was suggested that DIC was responsible for thrombocytopenia [7], but it was later shown that most patients with malaria do not have DIC [8]. A direct interaction between plasmodium and platelets has also been suggested, because *P. vivax* has been demonstrated by electron microscopy to exist inside the platelet of patients with vivax malaria [9]. The phagocytosis of platelets has also been reported in patients infected with malaria [10].

Immune mechanisms are considered to underlie the thrombocytopenia seen in malaria patients. Immune complexes that are present in the circulation of malaria-infected patients may play a role in the peripheral destruction of platelets as well as RBC [11,12]. Kelton et al. [13] reported that thrombocytopenia was present in 17 of 28 malaria patients, and the PAIgG level was increased in 16 cases, and they also demonstrated that the PAIgG level and platelet count returned to normal as the parasites were cleared from the circulation. They also confirmed that specific IgG bind directly to malaria antigen on platelets through the Fab terminus, and that the non-specific binding of IgG to platelet and immune complexes binding to Fc receptor of platelets are unlikely to be responsible for the thrombocytopenia in malaria. $CD4^+$ T-cells are also considered to be associated with the thrombocytopenia of a mouse malaria model [14].

In our two patients, laboratory investigations indicated no evidence of DIC, the numbers of megakaryocyte in their bone marrow were within normal range, and only mild splenomegaly was observed by ultrasonography. No phagocytosis of platelets was observed in the blood smear, the lymphocyte numbers were not increased, and immune complexes (C1q) were within normal range. The PAIgG levels were increased and showed a close inverse relationship with the thrombocytopenia. The PAIgG increase may be based on an increase in total platelet IgG, which reflects the α-granule IgG content of platelets, or platelet surface IgG, which reflect greater binding of immunoglobulin [15] such as antiplatelet antibody, immune complexes, non-specific IgG, or antimalarial antibody. Although there is no data about platelet surface IgG in present study, antiplatelet IgG is not a likely factor, because the PAIgG decreased without immunosuppressive therapy such as steroid hormone. Immune complexes and non-specific IgG are not likely because the patients immune complex levels were normal, and because of the above-described data of Kelton et al. [13]. Thus, antimalarial antibody is the most likely causal factor, because malaria antigen is present on the platelets [9]. The detailed mechanisms of the thrombocytopenia in our patients are unclear, but immunological mechanisms are suspected.

Furthermore, in Case 1, before the antimalarial antibody became positve in her serum, the PAIgG level increased and severe thrombocytopenia occurred. It is reported that antimalarial antibodies are first detected a few days after infection of the blood, and the antibody levels rise quickly to a plateau and are then maintained in serum for a long period of time [4]. Thus, it is likely that antimalarial antibody is not detected in serum because of its binding to platelets. The present finding that the thrombocytopenia and PAIgG increase occurred before the detection of antimalarial antibody is of note for the elucidation of thrombocytopenia in malaria infection.

CONCLUSION

We report here two cases of vivax malaria in which thrombocytopenia and an increase of PAIgG were closely associated and present before the detection of antimalarial antibody.

REFERENCES

1. Takeuchi T: Imported parasitic disease-recent epidemiology and progress in the chemotherapy. Jpn J Clin Med 40:2824, 1992.
2. Looareesuwan S, Davis JG, Allen DL, Lee SH, Bunnag D, White NJ: Thrombocytopenia in malaria. Southeast Asian J Trop Med Public Health 23:44, 1992.
3. World Health Organization. Malaria Action Programme: Severe and complicated malaria. Trans R Soc Trop Med Hyg 80(Suppl 1):1, 1986.
4. Voller A, Draper CC: Immunodiagnosis and seroepidermiology of malaria. Br Med Bull 38:173, 1982.
5. Kanou S, Waki S, Igarashi I, Nakazawa S, Masuda G, Suzuki M: Retrospective malaria diagnosis by indirect fluorescent antibody titration on Japanese patients. Jpn J Parasitol 39:475, 1990.
6. Horstman RD, Dietrich M, Bienzle U, Rasche H: Malaria-induced thrombocytopenia. Blut 42:157, 1981.
7. Dennis LH, Eichelberger JW, Inman MM, Conrad ME: Depletion of coagulation factors in drug-resistant *Plasmodium falciparum* malaria. Blood 29:713, 1967.
8. Shudowitzs RB, Karz J, Lurie A, Levin J, Metz J: Mechanisms of thrombocytopenia in malignant tertian malaria. Br Med J 2:515, 1973.
9. Fajardo LF, Tallent C: Malarial parasites within human platelets. JAMA 229:1205, 1974.
10. Jaff MS, Mckenna D, Mccann SR: Platelet phagocytosis: A probable mechanism of thrombocytopenia in *Plasmodium falciparum* infection. J Clin Pathol 38:1318, 1985.
11. Contreras C, June CH, Perrin LH, Lambert PH: Immunopathological aspects of *Plasmodium berhei* infection in five strains of mice. Clin Exp Immunol 42:403, 1980.
12. Perrin LH, Mackey LJ, Miescher PA: The hematology of malaria in man. Semin Hematol 19:70, 1982.
13. Kelton JG, Keystone J, Moore J, Denomme G: Immune-mediated thrombocytopenia of malaria. J Clin Invest 71:832, 1983.
14. Grau GE, Piguet PF, Gretener D, Vesin C, Lambert PH: Immunopathology of thrombocytopenia in experimental malaria. Immunology 65:501, 1988.
15. George JN: Platelet immunoglobulin G: Its significance for the evaluation of thrombocytopenia and for understanding the origin for α-granule proteins. Blood 76:859, 1990.